Supplementary Information

Establishing a yeast-based screening system for discovery of human GLUT5 inhibitors and activators

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Supplementary Table S1: Genotype of the *hxt⁰* strain

Strain name	Relevant genotype
EBY.VW4000	*MATa leu2-3,112 ura3-52 trp1-289 his3-1 MAL2-8c SUC2 Δhxt1-17 Δgal2 Δstl1::loxP Δagt1::loxP Δmph2::loxP Δmph3::loxP*

Supplementary Table S2: Primers used in this study

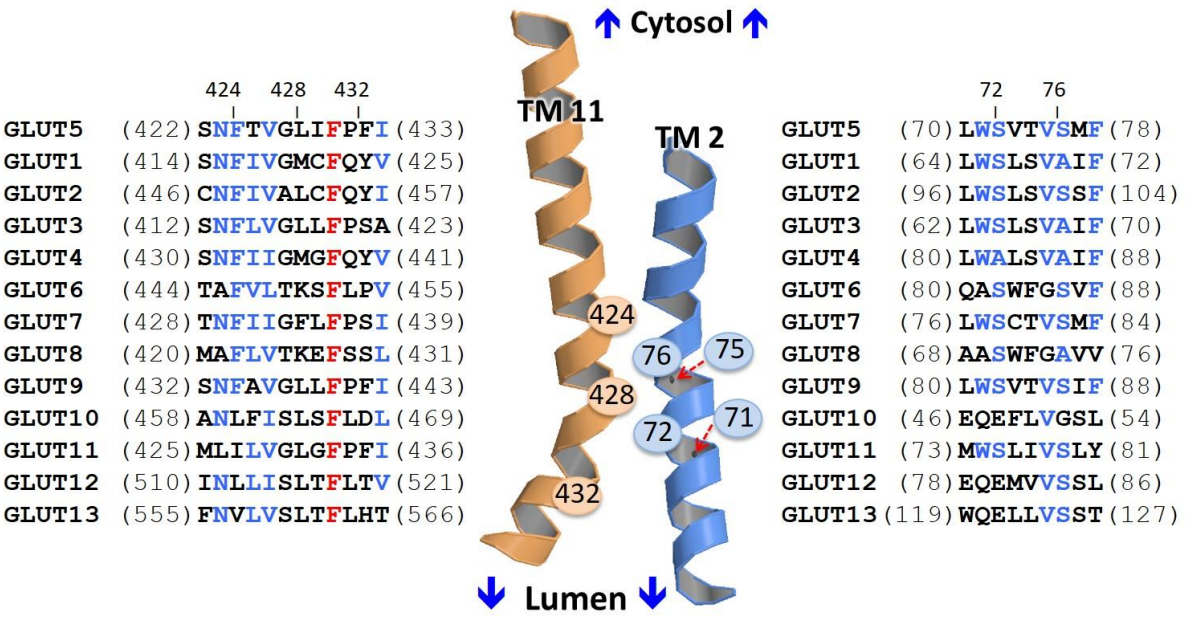
Template DNA	**Primer Name**	**Sequence (5' - 3')**	**Application**
GLUT5tr	MOP329	GATACAATTCTATTACCCCCATCCATACTCTAGAAATGAAAGAAGGTC GTCTGAC	forward primer for gap-repair cloning into p426MET25
	MOP330	ATGTAAGCGTGACATAACTAATTACATGACTCGAGTTATTGTTCAGAG GTCACCG	reverse primer for gap-repair cloning into p426MET25 and pRS72K
	MOP331	ATTACCCCCATCCATACTCTAGAAATGGAACAACAAGACCAATCTATG AAAGAAGGTCGTCTGAC	forward primer for introduction of 7 N-terminal amino acids into GLUT5tr and gap-repair cloning into p426MET25
	MOP370	ACAAAAACAAAAAGTTTTTTTAATTTTAATCAAAAAATGAAAGAAGGT CGTCTGAC	forward primer for gap-repair cloning into pRS72K
sGFP	MOP441	CTGAAAGAACTGCCGCCGGTGACCTCTGAACAAAGTAAAGGAGAAGAA CTTTTCACTG	forward primer for fusion of sGFP with the C-terminus of GLUT5tr variants
	JTP97	AATGTAAGCGTGACATAACTAATTACATGATTATTTGTAGAGCTCATC CATGC	reverse primer for gap-repair cloning into p426MET25

Supplementary Table S3: ORF sequences of GLUT5 variants

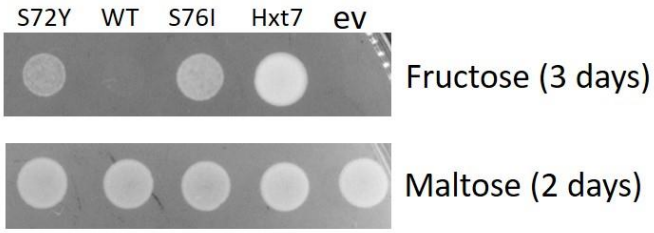
GLUT5 variant	ORF sequence
GLUT5tr	ATGAAAGAAGGTCGTCTGACCCTGGTTCTGGCTCTGGCTACCCTGATCGCCGCCTTCGGT TCTTCTTTCCAATACGGTTACAACGTGGCTGCTGTTAACAGCCCGGCTCTGCTGATGCAA CAGTTCTATAACGAGACCTATTACGGTCGCACCGGTGAGTTCATGGAAGATTTCCCACTG ACCCTGCTGTGGTCTGTTACTGTTAGCATGTTCCCGTTCGGCGGTTTCATTGGTAGCCTG CTGGTTGGTCCACTGGTGAACAAGTTCGGTCGCAAGGGTGCTCTGCTGTTCAACAACATT TTCAGCATTGTTCCGGCTATTCTGATGGGTTGTTCTCGCGTTGCTACCTCCTTCGAGCTG ATCATTATTTCTCGTCTGCTGGTTGGTATTTGCGCCGGCGTCAGCAGCAACGTTGTGCCA ATGTATCTGGGTGAACTGGCTCCAAAGAACCTGCGTGGTGCTCTGGGTGTTGTTCCACAG CTGTTCATCACCGTTGGCATTCTGGTTGCTCAGATTTTCGGTCTGCGTAACCTGCTGGCT AACGTTGACGGTTGGCCAATCCTGCTGGGTCTGACTGGTGTTCCAGCTGCTCTGCAACTG CTGCTGCTGCCATTCTTCCCGGAATCTCCGCGTTACCTGCTGATCCAGAAAAAAGACGAA GCCGCCGCCAAGAAAGCTCTGCAGACTCTGCGTGGTTGGGATTCCGTTGACCGTGAAGTT GCTGAAATTCGCCAGGAAGATGAAGCTGAAAAAGCTGCTGGTTTCATCTCTGTTCTGAAG CTGTTCCGTATGCGTAGCCTGCGTTGGCAGCTGCTGAGCATCATCGTTCTGATGGGTGGT CAACAGCTGAGCGGTGTTAACGCTATCTACTATTATGCTGATCAGATCTACCTGTCTGCT GGCGTTCCGGAAGAGCATGTCCAGTATGTTACCGCTGGCACTGGTGCTGTTAACGTTGTT ATGACTTTCTGCGCTGTTTTCGTTGTCGAACTGCTGGGTCGTCGTCTGCTGCTGCTGCTG GGTTTCTCTATCTGCCTGATCGCTTGCTGCGTTCTGACTGCTGCTCTGGCTCTGCAGGAT ACCGTTTCTTGGATGCCGTATATTTCTATCGTTTGCGTGATTTCTTACGTTATCGGTCAC GCTCTGGGTCCAAGCCCGATCCCAGCTCTGCTGATCACCGAGATCTTCCTGCAGTCTAGC CGTCCGAGCGCTTTCATGGTTGGTGGTTCTGTTCACTGGCTGTCTAACTTCACCGTTGGT CTGATCTTCCCGTTCATCCAGGAAGGTCTGGGTCCATATTCCTTCATCGTGTTCGCCGTT ATCTGTCTGCTGACCACCATTTACATCTTCCTGATCGTGCCAGAGACCAAGGCCAAGACC TTCATCGAGATCAACCAAATCTTCACCAAGATGAACAAAGTGAGCGAGGTTTACCCGGAA AAAGAGGAGCTGAAAGAACTGCCGCCGGTGACCTCTGAACAATAA
GLUT5	ATGGAACAACAAGACCAATCTATGAAAGAAGGTCGTCTGACCCTGGTTCTGGCTCTGGCT ACCCTGATCGCCGCCTTCGGTTCTTCTTTCCAATACGGTTACAACGTGGCTGCTGTTAAC AGCCCGGCTCTGCTGATGCAACAGTTCTATAACGAGACCTATTACGGTCGCACCGGTGAG TTCATGGAAGATTTCCCACTGACCCTGCTGTGGTCTGTTACTGTTAGCATGTTCCCGTTC GGCGGTTTCATTGGTAGCCTGCTGGTTGGTCCACTGGTGAACAAGTTCGGTCGCAAGGGT GCTCTGCTGTTCAACAACATTTTCAGCATTGTTCCGGCTATTCTGATGGGTTGTTCTCGC GTTGCTACCTCCTTCGAGCTGATCATTATTTCTCGTCTGCTGGTTGGTATTTGCGCCGGC GTCAGCAGCAACGTTGTGCCAATGTATCTGGGTGAACTGGCTCCAAAGAACCTGCGTGGT GCTCTGGGTGTTGTTCCACAGCTGTTCATCACCGTTGGCATTCTGGTTGCTCAGATTTTC GGTCTGCGTAACCTGCTGGCTAACGTTGACGGTTGGCCAATCCTGCTGGGTCTGACTGGT GTTCCAGCTGCTCTGCAACTGCTGCTGCTGCCATTCTTCCCGGAATCTCCGCGTTACCTG CTGATCCAGAAAAAAGACGAAGCCGCCGCCAAGAAAGCTCTGCAGACTCTGCGTGGTTGG GATTCCGTTGACCGTGAAGTTGCTGAAATTCGCCAGGAAGATGAAGCTGAAAAAGCTGCT GGTTTCATCTCTGTTCTGAAGCTGTTCCGTATGCGTAGCCTGCGTTGGCAGCTGCTGAGC ATCATCGTTCTGATGGGTGGTCAACAGCTGAGCGGTGTTAACGCTATCTACTATTATGCT GATCAGATCTACCTGTCTGCTGGCGTTCCGGAAGAGCATGTCCAGTATGTTACCGCTGGC ACTGGTGCTGTTAACGTTGTTATGACTTTCTGCGCTGTTTTCGTTGTCGAACTGCTGGGT CGTCGTCTGCTGCTGCTGGGTTTCTCTATCTGCCTGATCGCTTGCTGCGTTCTGACT GCTGCTCTGGCTCTGCAGGATACCGTTTCTTGGATGCCGTATATTTCTATCGTTTGCGTG ATTTCTTACGTTATCGGTCACGCTCTGGGTCCAAGCCCGATCCCAGCTCTGCTGATCACC GAGATCTTCCTGCAGTCTAGCCGTCCGAGCGCTTTCATGGTTGGTGGTTCTGTTCACTGG CTGTCTAACTTCACCGTTGGTCTGATCTTCCCGTTCATCCAGGAAGGTCTGGGTCCATAT TCCTTCATCGTGTTCGCCGTTATCTGTCTGCTGACCACCATTTACATCTTCCTGATCGTG CCAGAGACCAAGGCCAAGACCTTCATCGAGATCAACCAAATCTTCACCAAGATGAACAAA GTGAGCGAGGTTTACCCGGAAAAAGAGGAGCTGAAAGAACTGCCGCCGGTGACCTCTGAA CAATAA

Supplementary Table S4: Plasmids used in this study

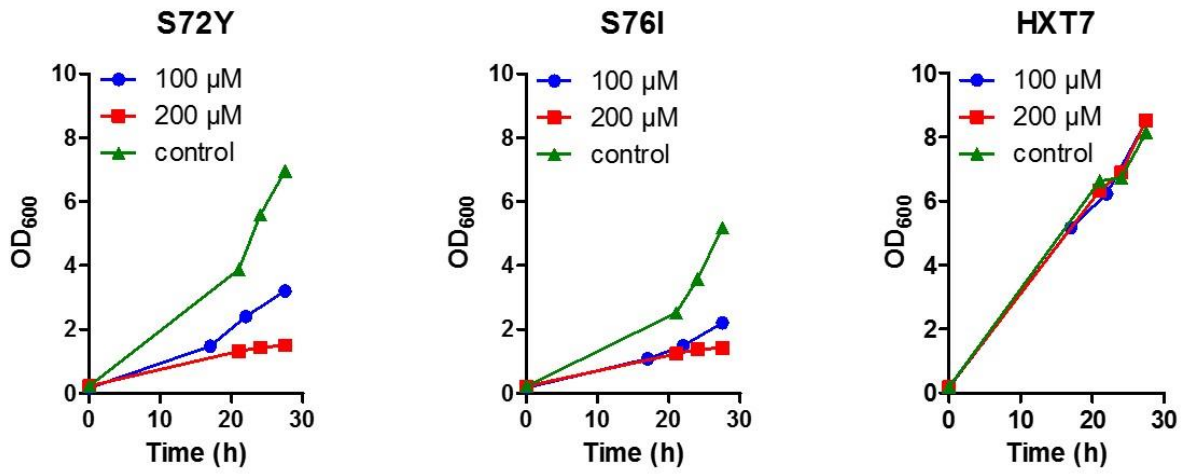
Plasmid name	Relevant properties and references
p426MET25	2µ origin; *URA3* marker; methionine-repressible MET25 promoter[1]
pRS72K	2µ origin; *TEF* promoter controlling *kanMX4* was exchanged by *TDH3* promoter in the pRS42K[2] backbone. A cassette comprising truncated *HXT7* promoter, multiple cloning site and *CYC1* terminator was integrated for heterologous gene expression. This cassette was amplified from the p426HXT7 vector[3]



Supplementary Figure S5: Localization of mutations in the TM domains of GLUT5. Shown is an alignment of relevant amino acid sequences (TM11, left and TM2, right) of GLUT1-GLUT13. The degree of conservation is indicated by the color code (none, black; moderate, blue; strict, red). The residues, which were mutated in yeast-expressed transporters, are S72, S76 of GLUT5 and W65, V69 of GLUT1. The positions of S72 and S76 as well those of interacting residues in TM11 (F424, L428 and F432) are shown in a model of TM11 and TM2 of GLUT5.



Supplementary Figure S6: Growth of EBY.VW4000 expressing GLUT5 variants on fructose and maltose. Serial dilutions of cells transformed with plasmids encoding GLUT5 variants (wild-type GLUT5tr; GLUT5tr^{S76I}; GLUT5tr^{S72YT}) were dropped onto indicated media. Empty vector (ev) was used as a negative control and a plasmid encoding the endogenous high-affinity hexose transporter Hxt7 as a positive control for growth on fructose. Maltose is shown as a viability control of the transformants. The plates were incubated at 30°C for two or three days.



Supplementary Figure S7: Inhibition of GLUT5 expressed in yeast cells by ECG. The EBY.VW4000 cells transformed with plasmids encoding GLUT5tr^{S72Y}, GLUT5tr^{S76I} or Hxt7 were cultivated in YEP media containing 2% (w/v) fructose and 200 µg/ml of G418 for plasmid selection. ECG was added at indicated concentrations or omitted (control). The growth was monitored over time by measuring OD_{600nm} of the culture. The results represent one measurement.

Supplementary References

1. Mumberg, D., Müller, R. & Funk, M. Regulatable promoters of Saccharomyces cerevisiae: comparison of transcriptional activity and their use for heterologous expression. *Nucleic Acids Res.* **22,** 5767–5768 (1994).
2. Taxis, C. & Knop, M. System of centromeric, episomal, and integrative vectors based on drug resistance markers for Saccharomyces cerevisiae. *BioTechniques* **40,** 73–78 (2006).
3. Hamacher, T., Becker, J., Gardonyi, M., Hahn-Hagerdal, B. & Boles, E. Characterization of the xylose-transporting properties of yeast hexose transporters and their influence on xylose utilization. *Microbiology* **148,** 2783–2788 (2002).